PROTOCOL TO MEASURE ACTIVITY OF SmtA AND Zur PROTEINS

1. Make a 5 mL pre-inoculation of DH5a :: pJ23101+B0030+SmtA+B0015 or DH5a :: pJ23101+B0030+Zur+B0015 using lysogenic broth (LB) containing 10 µL of 35mg/mL Cloramphenicol. Incubate overnight at 37°C under shaking (200 rpm).

2. Add to a 50 mL the following mixes as shown in table, allied to cloramphenicol (ratio 1 µL:mL of medium).

Tube	[$ZnCl_2$]	10 mM $ZnCl_2$	LB 2X	H_20	Final Volume
A	0 mM	0 mL	14 mL	0 mL	14 mL
B	0,2 mM	0,28 mL	7 mL	6,72 mL	14 mL
C	0,3 mM	0,42 mL	7 mL	6,58 mL	14 mL
D	0,5 mM	0,7 mL	7 mL	6,3 mL	14 mL
E	1 mM	1,4 mL	7 mL	5,6 mL	14 mL
F	2 mM	2,8 mL	7 mL	4,2 mL	14 mL
G	3 mM	4,2 mL	7 mL	2,8 mL	14 mL
H	5 mM	7 mL	7 mL	0 mL	14 mL

3. Aliquot 1 mL of mixture and 120 µL of pre-inoculation to a deep well plate, recording the positions in triplicate for each concentration at minimum. Each concentration should possess three wells per time sampling.

4. Incubate plates at 37°C under 200 rpm, collect samples of 1mL to optical density measurements at 600 nm each hour (We suggest use 8 concentrations and 4 different times per plate).

5. Use as control a plate containing DH5a :: psb1c3:mRFP. As blank use crude sterile medium available from the original tubes.